AMENDMENTS TO THE CLAIMS

The following is the status of the claims of the above-captioned application, as amended.

1. (Previously presented) A method for producing non-2μm-family plasmid protein comprising:

(a) providing a host cell comprising a 2μm-family plasmid, the plasmid comprising a gene encoding protein comprising the sequence of a chaperone protein and a gene encoding a non-2μm-family plasmid protein;

(b) culturing the host cell in a culture medium under conditions that allow the co-expression of the gene encoding protein comprising the sequence of the chaperone protein and the gene encoding a non-2μm-family plasmid protein; and

(c) purifying the thus expressed non-2μm-family plasmid protein from the cultured host cell or the culture medium.

2. (Original) The method of Claim 1 further comprising the step of formulating the purified non-2μm-family plasmid protein with a carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.

3. (Previously presented) A method of producing a non-2μm-family plasmid protein comprising:

a) providing a host cell comprising a 2μm-family plasmid, the plasmid comprising a gene encoding protein comprising the sequence of a chaperone protein and a gene encoding a non-2μm-family plasmid protein: and

b) culturing the host cell in a culture medium under conditions that allow the co-expression of the gene encoding protein comprising the sequence of the chaperone protein and the gene encoding a non-2µm-family plasmid protein.

wherein the non-2µm-family is a fungal (preferably yeast) or vertebrate non-2µm-family plasmid protein.

4. (Original) A 2µm-family plasmid comprising a gene encoding a protein comprising the sequence of a chaperone protein and a gene encoding a non-2µm-family plasmid protein, wherein if the plasmid is based on the 2µm-plasmid then it is a disintegration vector.

5. (Previously presented) A method according to claim 1 wherein the chaperone has a sequence of a fungal chaperone (preferably a yeast chaperone) or a mammalian chaperone (preferably a human chaperone).

6. (Previously presented) A method according to claim 1 wherein the chaperone comprises the sequence of a protein encoded by any one of *AHA1, CCT2, CCT3, CCT4, CCT5, CCT6, CCT7, CCT8, CNS1, CPR3,CPR6, EPS1, EPO1, EUG1, FMO1, HCH1, HSPIO, HSP12, HSP104, HSP26, HSP30, HSP42, HSP60, HSP78, HSP82, JEMI, MDJI, MDJ2, MPD1, MPD2, PDI1, PFD1, ABCI, APJ1, ATP11, A TPI2, BIT1, CDC37, CPR7, HSC82, KAR2, LHS1, MGE1, MRS11, NOB1, ECM1O, SSA1, SSA2, SSA3, SSA4, SSC1, SSE2, SIL1, SLS1, UBI4, ORM1, ORM2, PER1, PTC2, PSE1* and *HAC1* or truncated intronless *HAC1.*

7. (Previously presented) A method according to claim 1 wherein the chaperone is protein disulphide isomerase, or comprises the sequence of a protein encoded by *PSE1, ORM2* or *SSA1* or a variant or fragment thereof.

8. (Previously presented) A method according to Claim 1 wherein the host cell also expresses a second recombinant gene encoding a chaperone that is different to the first chaperone encoded by the plasmid.

9. (Original) A method according to Claim 8 wherein the second recombinant gene encoding a chaperone is chromosomally integrated.

10. (Previously presented) A method according to Claim 1 wherein the plasmid comprises two different genes encoding different chaperones, one of which gene is the second recombinant gene encoding a chaperone as defined by Claim 8.

11. (Previously presented) A method according to Claim 8 wherein one of the chaperones is protein disulphide isomerase.

12. (Previously presented) A method according to Claim 8 wherein one of the-chaperones is ORM2.

13. (Previously presented) A method according to Claim 8 wherein the two chaperones are protein disulphide isomerase and ORM2.

14. (Previously presented) A method according to claim 1wherein the non-2μm-family plasmid protein comprises a leader sequence effective to cause secretion in yeast.

15. (Previously presented) A method according to claim 1 wherein the non-2μm-family plasmid protein is a eukaryotic protein, or a fragment or variant thereof, preferably a vertebrate or a fungal (such as a yeast) protein.

16. (Previously presented) A method according to claim 1 wherein the non-2μm-family plasmid protein is a commercially useful protein.

17. (Previously presented) A method according to claim 1 wherein the non-2μm-family plasmid protein comprises a sequence selected from albumin, a monoclonal antibody, an etoposide, a serum protein (such as a blood clotting factor), antistasin, a tick anticoagulant peptide, transferrin, lactoferrin, endostatin, angiostatin, collagens, immunoglobulins, or Immunoglobulin-based molecules or fragment of either (e.g. a dAb, Fab' fragments, F $(ab')_2$, scAb, scFv or scFv fragment), a Kunitz domain protein interferons, interleukins, IL 10, IL 11, IL2, interferon α species and sub-species, interferon β species and sub-species interferon *γ* species and sub-species, leptin, CNTF, CNTF $_{Ax15}$ (Axokine™), ILl-receptor antagonist, erythropoietin (EPO) and EPO mimics, thrombopoietin (TPO) and TPO mimics, prosaptide, cyanovirin-N, 5-helix, T20 peptide, T1249 peptide, HIV gp41, HIV gp120, urokinase, prourokinase, tPA, hirudin, platelet derived growth factor, parathyroid hormone, proinsulin, insulin, glucagon, glucagon-like peptides, insulin-like growth factor, calcitonin, growth hormone, transforming growth factor β, tumour necrosis factor, G-CSF, GM-CSF, M-CSF, FGF, coagulation factors in both pre and active forms, including but not limited to plasminogen, fibrinogen, thrombin, pre- thrombin, pro-thrombin, von Willebrand's factor, α-antitrypsin, plasminogen activators, Factor VII, Factor VIII, Factor IX, Factor X and Factor XIII, nerve growth factor, LACI, platelet-derived endothelial cell growth factor (PD-ECGF), glucose oxidase, serum cholinesterase, aprotinin, amyloid precursor protein, inter-alpha trypsin inhibitor, antithrombin III, apolipoprotein species, Protein C, Protein S, or a variant or fragment of any of the above.

18. (Previously presented) A method according to claim 1 wherein the non-2μm-family plasmid protein comprises the sequence of albumin or a variant or fragment thereof.

19. (Previously presented) A method according to claim 1 wherein the non-2μm-family plasmid protein comprises the sequence of a transferrin family member, preferably transferrin or lactoferrin, or a variant or fragment thereof.

20. (Previously presented) A method according to claim 1 wherein the non-2μm-family plasmid protein comprises a fusion protein, such as a fusion protein of albumin or a

transferrin family member or a variant or fragment of either, fused directly or indirectly to the sequence of
another protein.

21. (Previously presented) A host cell comprising a plasmid as defined by claim 4.

22. (Original) A host cell according to Claim 21 wherein a chaperone encoded by the plasmid is an essential gene.

23. (Original) A host cell according to Claim 22 wherein, in the absence of the plasmid, the host cell does not produce the chaperone.

24. (Currently amended) A host cell according to ~~Claims~~ Claim 21, wherein the host cell is a yeast cell.

25. (Original) A host cell according to Claim 24 in which the plasmid is based on pSRI, pSB3 or pSB4 and the yeast cell is *Zygosaccharomyces rouxii,* the plasmid is based on pSB 1 or pSB2 and the yeast cell is *Zygosaccharomyces bailli,* the plasmid is based on pSM1 and the yeast cell is *Zygosaccharomyces fermentati,* the plasmid is based on pKDI and the yeast cell is *Kluyvejomyces drosophilarum,* the plasmid is based on pPMI and the yeast cell is *Pichia menabranaefaciens,* or the plasmid is based on the 2µm plasmid and the yeast cell is *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis.*

26. (Original) A host cell according to Claim 25 in which the plasmid is based on the 2µm plasmid and the yeast cell is *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis.*

27. (Previously presented) A method according to Claim 1 wherein the host cell is a host cell as defined by Claim 21.

28. (Previously presented) A method according to Claim 27 wherein the host cell is a host cell as defined by Claim 23.

29. (Original) A method according to Claim 27 wherein the step (b) involves culturing the host cell in non-selective media, such as a rich media.

30. (Currently amended) A method for producing non-2μm-family plasmid protein comprising:

(a) providing a host cell comprising a first recombinant gene encoding a protein comprising the sequence of a first chaperone protein, a second recombinant gene encoding a protein comprising the sequence of a second chaperone protein and a third recombinant gene encoding a non-2μm-family plasmid protein, wherein the first and second chaperones are different;

(b) culturing the host cell in a culture medium under conditions that allow the expression of the first, second and third genes; and

(c) optionally purifying the thus expressed non-2μm-family plasmid protein from the cultured host cell or the culture medium; and .

(d) optionally, ~~lyophilising~~lyophilizing the thus purified protein.

31. (Original) The method of Claim 30 further comprising the step of formulating the purified non-2μm-family plasmid protein with a carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.

32. (Previously presented) A method according to Claim 30 wherein the first and second chaperones comprise the sequence of a protein encoded by any one of *AHA1, CCT2, CCT3, CCT4, CCT5, CCT6, CCT7, CCT8, CNS1, CPR3,CPR6, EPS1, EPO1, EUG1,*

FMO1, HCH1, HSPIO, HSP12, HSP104, HSP26, HSP30, HSP42, HSP60, HSP78, HSP82, JEMI, MDJI, MDJ2, MPD1, MPD2, PDI1, PFD1, ABCI, APJ1, ATP11, A TPI2, BIT1, CDC37, CPR7, HSC82, KAR2, LHS1, MGE1, MRS11, NOB1, ECM1O, SSA1, SSA2, SSA3, SSA4, SSC1, SSE2, SIL1, SLS1, UBI4, ORM1, ORM2, PER1, PTC2, PSE1 and *HAC1* or truncated intronless *HAC1.*

33. (Previously presented) A method according to Claim 30 wherein the first chaperone is protein disulphide isomerase.

34. (Previously presented) A method according to Claim 30 wherein the second chaperone is ORM2.

35. (Previously presented) A method according Claim 30 wherein at least one of the first or second chaperones is encoded by a chromosomally integrated recombinant gene.

36. (Previously presented) A method according Claim 30 wherein at least one of the first or second chaperones is encoded by a gene on a plasmid.

37. (Previously presented) A method according to Claim 36 wherein the plasmid is a plasmid as defined by Claim 4.

38. (Original) A host cell comprising a first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) and a second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein.

39. (Canceled)

40. (Previously presented) A host cell according to Claim 38 wherein the transferrin-based protein comprises the sequence of transferrin or any other member of the

transferrin family (e.g. lactoferrin), a variant or fragment thereof or a fusion protein comprising transferrin, a variant or fragment thereof.

41. (Previously presented) A host cell according to Claim 38 wherein the first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) is provided on a plasmid.

42. (Previously presented) A host cell according to Claim 41 wherein the plasmid is a 2μm-family plasmid.

43. (Previously presented) A host cell according to Claim 38 wherein the first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) is chromosomally integrated.

44. (Previously presented) A host cell according to Claim 43 wherein the first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) is chromosomally integrated at the locus of an endogenously encoded PDI gene, preferably without disrupting the expression of the endogenous PDI gene.

45. (Previously presented) A host cell according to Claim 38 wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is provided on a plasmid.

46. (Previously presented) A host cell according to Claim 45 wherein the plasmid is a 2μm-family plasmid.

47. (Previously presented) A host cell according to Claim 38 wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is chromosomally integrated.

48. (Previously presented) A host cell according to Claim 47 wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is chromosomally integrated at the locus of an endogenously encoded PDI gene, preferably without disrupting the expression of the endogenous PDI gene.

49. (Original) A method for producing non-2μm-family plasmid protein comprising:

(a) providing a host cell comprising a first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof and a second recombinant gene encoding a non-2μm-family plasmid protein; and

(b) culturing the host cell in a culture medium under conditions that allow the expression of the first and second genes.

50. (Original) The method of Claim 49 further comprising the step of formulating the purified non-2μm-family plasmid protein with a carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.

51. (Previously presented) A method according to Claim 49 wherein the first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof is integrated into the chromosome of the host cell.

52. (Previously amended) A method according to Claim 49 wherein the first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof is located on a plasmid.

53. (Original) A host cell comprising first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof and a second recombinant gene encoding a non-2μm-family plasmid protein.

54. (Canceled)

55. (Original) A plasmid comprising a first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof and a second recombinant gene encoding a non-2μm-family plasmid protein.

56. (Original) A plasmid according to Claim 55 which is a 2μm-family plasmid.

57 . (Previously presented) A method according to Claim 49 wherein the non-2μm-family plasmid protein is as defined in Claim 14.

58. (Previously presented) A host cell comprising a plasmid, the plasmid comprising a gene that encodes an essential chaperone wherein, in the absence of the plasmid, the host cell is unable to produce the chaperone, the plasmid further comprising a recombinant gene encoding a non-μm-family plasmid protein, such as a non-2μm-family plasmid protein as defined in Claim 14.

59. (Original) A host cell according to Claim 58 wherein, in the absence of the plasmid, the host cell is inviable.

60. (Previously presented) The host cell of Claim 58 wherein the chaperone is protein disulphide isomerase.

61. (Original) A plasmid comprising, as the sole selectable marker, a gene encoding an essential chaperone.

62. (Previously presented) The plasmid of Claim 61 further comprising a gene encoding a non- μm-family plasmid protein, such as a non-2μm-family plasmid protein as defined in Claim 14.

63. (Previously presented) The plasmid of Claim 61 which is a 2μm-family plasmid.

64. (Previously presented) A method for producing a non-2μm-family plasmid protein comprising the steps of:

(a) providing a host cell as defined by Claim 58; and

(b) culturing the host cell in a culture medium under conditions that allow the expression of the essential chaperone and the non-2μm-family plasmid protein.

65. (Previously presented) The method of Claim 64 wherein the host cell comprises a plasmid as defined by Claim 61.

66. (Previously presented) The method of Claim 64 wherein step (b) is performed by culturing the host cell in a non-selective medium, such as a rich or complex medium.

67-68. (canceled)

69. (Currently amended) A method for increasing the expression of a non-2μm-family plasmid protein in a host cell comprising
providing a polynucleotide comprising a promoter operably connected to a coding sequence encoding a chaperone; and
expressing of the polynucleotide sequence within the host cell,
wherein the promoter is ~~characterised~~ characterized in that it achieves a lower level of expression of the chaperone than would be achieved if the coding sequence were to be operably connected to its naturally occurring promoter.

70. (Previously presented) A method for producing a non-2μm-family plasmid protein comprising the steps of:

(a) providing a host cell comprising a recombinant gene that the sequence of a promoter operably connected to a coding sequence encoding a chaperone, the promoter being ~~characterised~~ characterized in that it achieves a lower level of expression of the chaperone than would be achieved if the coding sequence were to be operably connected to its naturally occurring promoter, and the host cell further comprising a recombinant gene encoding a non-2μm-family plasmid protein;

(b) culturing the host cell ~~in a~~ under conditions that allow the expression of the chaperone and the non-2μm-family plasmid protein.

71. (Currently amended) The method of Claim 1 further comprising the step of lyophilizing ~~lyophilising~~ the thus purified protein.

72. (Previously amended) The method of Claim 49 further comprising the step of purifying the thus expressed non-2μm-family plasmid protein from the cultured host cell or the culture medium.

73 (Currently amended) The method of Claim 72 further comprising the step of ~~lyophilising~~lyophilizing
the thus purified protein.

74. (Previously presented) The method of Claim 72 further comprising the step of formulating the purified non-2μm-family plasmid protein with a carrier or diluent.

75. (Original) The method of Claim 74 further comprising the step of presenting the thus formulated protein in a unit dosage form.